Applic. No. 09/842,111

Amdt. dated May 26, 2004

Reply to Office Action of Feb. 27, 2004

Amendments to the Claims:

The listing of claims will replace all prior versions and listings, of claims in the application:

Listing of Claims:

Claims 1-5 (canceled)

Claim 6 (currently amended) A method of determining the relative level of Dihydropyrimidine

dehydrogenase (DPD) gene expression in a tissue sample comprising:

(a) obtaining a tumor sample from a patient;

(b) fixing at least a portion of said tumor sample in paraffin to achieve a fixed and paraffin

embedded (FPE) tumor tissue sample;

(c) isolating mRNA from said (FPE) tumor tissue sample;

(d) determining the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA isolated

from said FPE tumor tissue sample by amplifying the mRNA using an oligonucleotide

primer SEQ ID NO: 1 and oligonucleotide primer SEQ ID NO: 2; and

(e) comparing the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA from

step(d) to an amount of mRNA of an internal control gene.

Claims 7-9 (canceled)

Claim 10 (previously presented) The method of claim 6, wherein the RNA is isolated in the presence

of an effective amount of chaotropic agent.

Claim 11 (previously presented) The method of claim 6, wherein the tumor sample comprises non-

tumor tissue and tumor tissue.

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Claims 12-16 (canceled)

Claim 17 (currently amended) A method of determining the relative level of Dihydropyrimidine

dehydrogenase (DPD) gene expression in a tissue sample comprising;

(a) obtaining a tumor sample from a patient;

(b) fixing at least a portion of said tumor sample in paraffin to achieve a fixed and paraffin

embedded (FPE) tumor tissue sample;

(c) isolating mRNA from said FPE tumor tissue sample;

(d) determining the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA isolated

from said FPE tumor tissue sample by amplifying the mRNA using an oligonucleotide

primer SEQ ID NO: 7 and oligonucleotide primer SEQ ID NO: 8; and

(e) comparing the amount of the mRNA from step(d) to an amount of mRNA of an internal

control.

Claims 18-19 (canceled)

Claim 20 (previously presented) The method of claim 17, wherein the mRNA isolated from said FPE

tumor tissue is isolated in the presence of an effective amount of chaotropic agent.

Claim 21 (canceled)

Claim 22 (previously presented) The method of claim 17, wherein a FPE tumor tissue sample

comprises non-tumor tissue and tumor tissue.

Claims 23-26 (canceled)

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Claim 27 (currently amended) A method of determining the relative level of *Dihydropyrimidine* dehydrogenase (DPD) gene expression in a tissue sample comprising:

- (a) obtaining a tumor sample from a patient, wherein said tumor sample is fixed and paraffin embedded (FPE);
- (b) isolating mRNA from said FPE tumor sample, wherein said FPE tumor sample is heated to a temperature in the range of about 50 to about 100°C;
- (c) determining the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA by amplifying the mRNA using an oligonucleotide primer SEQ ID NO: 1 and SEQ ID NO: 2; and
- (d) comparing the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA from step (c) to an amount of mRNA of an internal control gene.

Claim 28 (Currently amended) A method of determining the relative level of *Dihydropyrimidine* dehydrogenase (DPD) gene expression in a tissue sample comprising:

- (a) obtaining a tumor sample from a patient, wherein said tumor sample is fixed and paraffin embedded (FPE);
- (b) isolating mRNA from said FPE tumor sample, wherein said FPE tumor sample is heated to a temperature in the range of about 50 to about 100°C;
- (c) determining the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA by amplifying the mRNA using an oligonucleotide primer SEQ ID NO: 7,and SEQ ID NO: 8: and
- (d) comparing the amount of *Dihydropyrimidine dehydrogenase* (*DPD*) mRNA from step (c) to an amount of mRNA of an internal control gene.

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Claim 29 (previously presented) The method of claims 27 or 28, wherein said internal control gene is

β-actin.

Claim 30 (canceled)

Claim 31 (previously presented) The method of claims 27 or 28, wherein the mRNA is isolated in the

presence of an effective amount of chaotropic agent.

Claim 32 (presently amended) A method for determining the relative level of *Dihydropyrimidine*

dehydrogenase (DPD) gene expression in a fixed and paraffin embedded (FPE) tissue sample

comprising:

(a) deparaffinizing the FPE tissue sample to obtain a deparaffinized sample;

(b) isolating mRNA from the deparaffinized sample in the presence of an effective amount

of a chaotropic agent by first heating the tissue sample in a solution comprising an

effective concentration of a chaotropic compound to a temperature in the range of

about 75° to about 100° C for a time period of 5 to 120 minutes and recovering said

mRNA from said chaotropic solution; and-

(c) subjecting the mRNA to amplification using a pair of oligonucleotide primers capable of

amplifying a region of the DPD gene, to obtain an amplified sample; wherein the pair of

primers is primer pair SEQ ID NO:1 and 2 or primer pair SEQ ID NO: 7 and 8; and

(d) determining the quantity of DPD mRNA relative to the quantity of an internal control

gene's mRNA.

Claim 33 (previously presented) The method of claim 32 wherein, the internal control gene is \(\mathcal{B} \)-actin.

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Claim 34 (currently amended) A method for determining the relative level of *Dihydropyrimidine dehydrogenase* (DPD) gene expression in a fixed and paraffin embedded tissue sample comprising:

- (a) deparaffinizing the tissue sample to obtain a deparaffinized sample;
- (b) isolating mRNA from the deparaffinized sample by first heating the deparaffinized tissue sample in a solution comprising an effective concentration of a chaotropic agent to a temperature in the range of about 50° to about 100° C and recovering said mRNA from said solution; and
- (c) determining the quantity of DPD mRNA relative to the quantity of an internal control gene's mRNA.

Claim 35 (currently amended) A method for determining a platinum-based chemotherapeutic regimen for treating a tumor in a patient comprising:

- (a) obtaining a tissue sample of the tumor and fixing the sample to obtain a fixed tumor sample;
- (b) isolating mRNA from the fixed tumor sample, wherein the fixed tumor sample is heated in the presence of an effective amount of a chaotropic agent and wherein the heating occurs at a temperature from about 50° to about 100° C;
- (c) subjecting the mRNA to amplification using a pair of oligonucleotide primers capable of amplifying a region of the *Dihydropyrimidine dehydrogenase* (DPD) gene to obtain an amplified sample; wherein the pair of primers is primer pair SEQ ID NO:1 and 2 or primer pair SEQ ID NO: 7 and 8;
- (d) determining the amount of amplified DPD mRNA in the amplified sample;
- (e) comparing the amount of DPD mRNA from step (d) to an amount of mRNA of an internal control gene; and
- (f) determining a platinum-based chemotherapeutic regimen based on the amount of DPD

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mRNA in the amplified sample and a predetermined threshold level for DPD gene expression.

Claim 36 (canceled)

Claim 37 (currently amended) The method of claim 35 wherein the fixed tumor sample is heated in the presence of an effective amount of a chaotropic agent and wherein the heating occurs at a temperature from about 75 °C to about 100 °C for a period of about 5 to about 120 minutes.